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(U) Effects of Electric Fields on Membrane-Bound Na,K-ATPase

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The Na,K-ATPase of human erythrocytes can transport Na^+ and K^+ against their respective concentration gradient using the energy supplied by an a.c. electric field. It is a model for studying the interaction of an electric field and a cell at the molecular level. The a.c. activation has an optimal frequency and an optimal field strength and ATP hydrolysis is not required. We have obtained a complete set of kinetic data and used them to construct the electroconformational coupling model. Analysis shows that an a.c. field can enforce conformational oscillation of the enzyme, thus, enabling it to transduce energy or signals. The model provides an efficient mechanism for cell-to-cell, tissue-to-tissue, and organism-to-organism communications. It may also help us understand the broad biological effects of electromagnetic fields. Oscillating electric fields are also found to exert mechanical force on cell membranes. This phenomenon is under investigation.

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Progress Reports on ONR Grant N00014-88-J-1220

PRINCIPAL INVESTIGATOR: Tian Y. Tsong

CONTRACTOR: University of Minnesota

CONTRACT TITLE: Effect of Electric Fields on Membrane Bound Na,K-ATPase

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PROGRESS:

Our work last year focused on obtaining a complete set of kinetic data on the electric activation of Na,K-ATPase and to demonstrate that the field induced transports of Na^+ and K^+ were active transport and this activity did not require hydrolysis of ATP. These results are summarized in Table 1 and Table 2. In Table 1, it is shown that with a 20 V/cm, 1.0 kHz a.c. field, only the Rb uptake was stimulated (S - NS, 10.5 attomole/RBC-hr) and this activity was ouabain sensitive. When 20 V/cm, 1.0 MHz a.c. field was used, only ouabain sensitive Na efflux was stimulated (16.8 attomole/RBC-hr). These two activities were transport against their respective concentration gradient. Last year we reported that the conditions used above are optimal for the activation of the two pumps. Data given here show that these activities were specific to Na,K-ATPase and non-specific leaks (Na uptake, Rb efflux) were not stimulated by the a.c. fields.

Data in Table 2 indicate that the a.c. stimulated transport of Na ion did not require ATP, in the range 10 μM to 800 μM. A complete elimination of ATP was not practical because the ATP depleted cells quickly deteriorated before any experiment can be completed. 10 μM is much lower than the K_m of the ATP hydrolysis activity, which is in the 500 μM range. We conclude that the stimulated active transport of these ions did not require hydrolysis of ATP.

We also collaborated with Dr. Dean Astumian for the analysis of data using the electroconformational coupling model which we proposed and developed earlier. It is shown that the effects of an a.c. field is to enforce the conformational oscillation of the enzyme species that are involved in the catalytic processes. Figure 1 shows an example of such analysis.

WORK PLAN:

We will purify Na,K-ATPase, reconstitute it into lipid vesicles. Such a system will be used to verify the above results. Fluorescence method will be used to detect electroconformational changes of the enzyme when proteoliposomes are exposed in an a.c. field. We have also observed that an a.c. field of defined frequency and amplitude can cause deformations in unilamellar lipid vesicle. This phenomenon provide another mechanism for an electric field to interact with a cell membrane.

INVENTION: None.

Table 1 Electric Field Stimulated Active Transport of Rb^+ and Na^+ at 3.5°C

	Cell. Ion Conc.			Medium Ion Conc.				Measured Ion Movement						
	Na	K	Rb	Na	K	Rb	Mg	NS	S	ONS	OS	NS-ONS	S-OS	S-NS
	(mM)			(mM)				(amole/RBC-hr)				(amole/RBC-hr)		
20 V/cm a.c. 1.0 kHz														
Rb Influx	6	75	27	2.5	0	12.5	2	13.0 (0.3)	23.5 (1.2)	10.1 (0.6)	11.1 (0.15)	2.9 (0.6)	12.4 (1.2)	10.5[a] (1.2)
Rb Efflux	6	65	15	2.5	0	12.5	2	42.1 (1.7)	43.4 (1.1)	41.7 (1.5)	41.6 (1.5)	0.4 (1.7)	1.8 (1.5)	1.3 (1.7)
Na Influx	6	75	0	150	5	0	2	3.2 (<0.1)	3.54 (0.2)	4.0 (0.1)	6.2 (0.3)	-0.8 (0.1)	-2.7 (0.2)	0.4 (0.2)
Na Efflux	6	75	0	150	5	0	2	4.3 (2.0)	6.2 (0.6)	1.7 (0.1)	1.9 (0.8)	-1.9 (2.0)	4.3 (0.6)	1.9 (2.0)
20 V/cm a.c. 1.0 MHz														
Rb Influx	6	75	27	2.5	0	12.5	2	10.6 (3.8)	10.4 (3.5)	8.8 (1.8)	8.9 (1.6)	2.1 (3.8)	1.5 (3.5)	-0.5 (3.5)
Rb Efflux	6	65	15	2.5	0	12.5	2	38.3 (2.0)	37.7 (1.0)	40.4 (0.3)	39.5 (1.1)	-2.1 (2.0)	-1.8 (1.0)	-0.6 (2.0)
Na Influx	6	75	0	150	5	0	2	6.1 (0.6)	6.9 (0.9)	6.6 (0.3)	6.9 (<0.1)	-0.5 (0.6)	0.0 (0.9)	0.8 (0.9)
Na Efflux	6	75	0	150	5	0	2	4.0 (2.7)	20.8 (3.2)	2.0 (0.1)	5.3 (1.8)	2.0 (2.7)	15.5 (3.2)	16.8[a] (3.2)

For measurements of ion concentration by flame photometry and ion movement using radioactive tracers see Method. S, NS, ONS and OS denote, respectively, Stimulated, Non-Stimulated, Ouabain treated Non-Stimulated and Ouabain treated Stimulated samples. Each value is the mean of 3-5 measurements. Standard deviation is given in parenthesis. 1 amole = 1 atto-mole=$1x10^{-18}$ mole. 1 amole/RBC-hr = 0.018 mmole/liter cells-hr.

a). Values vary for erythrocyte samples from different individuals. Rb^+ influx values are in the range 10 - 20 amole/RBC-hr and Na^+ efflux values are in the range 15 - 30 amole/RBC-hr.

b). Data given in this table were from blood samples of a single individual.

c). In Na^+ influx and efflux experiments, Rb^+ was not added because our intention was only to demonstrate the active pumping of Na^+. K^+ was present on both sides of the membrane.

Table 2 Effect of Cytoplasmic ATP on Voltage Activation of Na^+ pumping mode

Sample	Temp	[ATP]	NS	S	ONS	OS	NS-ONS	S-ONS	S-NS	S-OS
	(°C)	(uM)	(attomole/RBC-hr)							
Fresh RBC	3.5	600-800	18.5 (2.2)	36.5 (1.2)	15.7 (2.1)	16.7 (1.8)	2.8 (2.1)	20.8 (2.1)	18.0 (2.2)	19.8 (1.8)
	26	600-800	24.4 (2.8)	44.8 (1.7)	16.4 (2.8)	15.2 (2.3)	8.0 (2.8)	28.5 (2.8)	20.5 (2.8)	29.6 (2.8)
ATP Depleted RBC	3.5	5-15	14.4 (0.2)	30.4 (3.0)	13.3 (2.8)	15.4 (1.3)	1.2 (2.8)	17.2 (3.0)	16.0 (3.0)	15.1 (3.0)
	26	5-15	20.2 (2.2)	43.3 (3.0)	17.4 (1.6)	15.4 (2.8)	2.8 (2.2)	25.9 (3.0)	23.1 (3.0)	27.9 (3.0)

Na^+ efflux was measured. The cytoplasmic concentration of Na^+ was 6 mM and of K^+ was 75 mM. The medium ion concentration was 150 mM for Na^+, 5 mM for K^+ and 2 mM for Mg^{++}.

Symbols used and other conditions are the same as those given in Table 1.

Data are from blood samples of one individual.

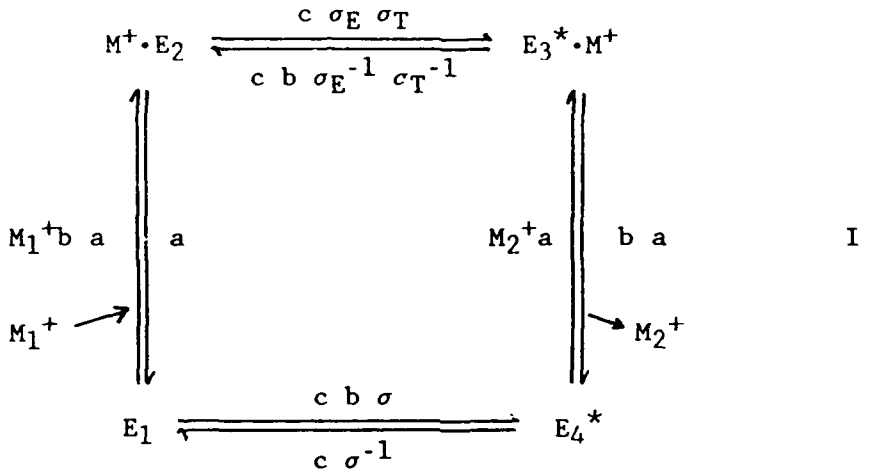
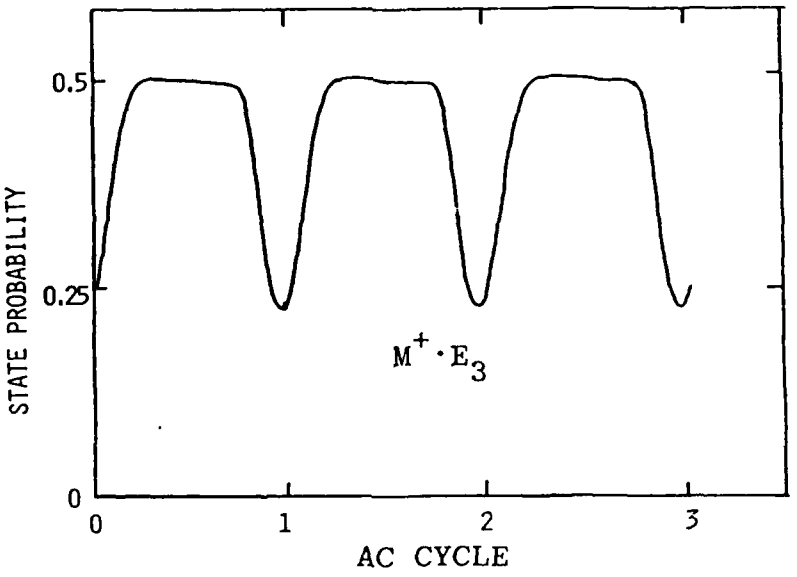


Fig. 1 Enforced Electroconformational Oscillations of Enzyme States. Computer analysis was done to demonstrate an a.c. induced conformational oscillation and the subsequent clockwise pumping of substrate, using the kinetic Scheme I shown in the text with these parameter values: b = 10, a = 1000 s^{-1}, c = 1000 s^{-1}, z_s = 0, and x ψ = -1. State probability of M^+E_2 as a function of the a.c. cycle. Other enzyme species also oscillate with the a.c. field.



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